ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, March 1978, p. 349-357
0066-4804/8/1303-0349$02.00/0

Vol. 13, No. 3
Printed in U.S.A.

In Vitro Activity of Piperacillin, a New Semisynthetic Penicillin with an Unusually Broad Spectrum of Activity

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Received for publication 16 August 1977

The in vitro activity of piperacillin (T-1220), a new semisynthetic derivative of aminobenzylpenicillin, was investigated. The majority of streptococci and pneumococci were inhibited by 0.12 μg/ml; the staphylococci and enterococci were inhibited by 2 μg/ml. Piperacillin was slightly more active against *Neisseria* and *Haemophilus influenzae* than was ampicillin. Piperacillin was active against all members of the *Enterobacteriaceae* including the *Klebsiella*, 58% of which were inhibited by 8 μg/ml. The activity of piperacillin was at least equivalent, but generally superior, to that of ampicillin or carbenicillin on species susceptible to these drugs. Most striking was its activity on *Pseudomonas aeruginosa*: 50% were inhibited by 2 μg/ml, and 83% were inhibited by 4 μg/ml. The minimum bactericidal concentrations were very close to the minimum inhibitory concentrations, and in most species only a slight inoculum effect was observed on the minimum bactericidal values except for certain *P. aeruginosa* strains. A complete parallel resistance exists between piperacillin and ampicillin or carbenicillin. However, the clinical importance of this is largely mitigated by the intrinsically higher activity of piperacillin.

Since the availability of ampicillin and carbenicillin, the spectrum of activity of the penicillins has been broadened to include gram-negative bacilli. The extensive use of these drugs has demonstrated that they were a major breakthrough in fulfilling the need for safe antibiotics against infections with gram-negative microorganisms. Nevertheless, the activity of ampicillin against *Enterobacteriaceae* is practically limited to *Escherichia coli, Proteus mirabilis, Salmonella,* and *Shigella,* whereas it remains ineffective at therapeutic concentrations against most indole-positive *Proteus* strains, against *Providencia, Klebsiella, Enterobacter,* and *Serratia,* and also against *Pseudomonas aeruginosa.* Carbenicillin partially fills the gap with its good activity at low concentrations against the indole-positive *Proteus* and *Providencia* and its relatively good activity against *Serratia marcescens* and *P. aeruginosa,* although most strains of the latter species are only susceptible at concentrations of 32 μg or more per ml. Piperacillin (T-1220), a semisynthetic aminobenzylpenicillin derivative developed by Toyama Chemical Co., Ltd., is the generic name for sodium 6-[D(−)-α-(4-ethyl-2,3-dioxo-1-piperazinylcarbonylamino)-α-phenylacetamido] penicillanate (2). The antibiotic has recently become available for investigation, and preliminary results showed a wide spectrum of activity (S. Mitsuhashi, I. Saikawa, and T. Yasuda, Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 16th, Chicago, Ill., Abstr. no. 349, 1976; Y. Ueda, A. Saito, J. Shimida, I. Saikawa, and T Yasuda, 16th ICAAC, Abstr. no. 350, 1976). This paper reports studies on the overall activity of piperacillin in comparison with other β-lactam antibiotics on a large number of gram-positive and gram-negative clinical isolates.

MATERIALS AND METHODS

Microorganisms. A total of 767 clinical isolates of bacteria were tested. After identification by standard criteria, these were distributed as follows: *E. coli* (60); *Klebsiella pneumoniae* (80); *Enterobacter* (49); *Citrobacter freundii* 15); *Citrobacter diversus* (12); *Salmonella* (30); *Serratia marcescens* (30); *Proteus mirabilis* (61); *Proteus vulgaris* (30); *Proteus morganii* (30); *Proteus rettgeri* (11); *Providencia* (20); *Pseudomonas aeruginosa* (60); *Pseudomonas maltophilia* (11); *Pseudomonas cepacia* (4); *Pseudomonas stutzeri* (6); *Pseudomonas putrefaciens* (6); *Pseudomonas fluorescens* (8); *Pseudomonas putida* (7); *Acinetobacter* (30); *Staphylococcus aureus* (penicillin susceptible) (30); *Streptococcus pyogenes* (14); *Streptococcus agalactiae* (15); *Streptococcus pneumoniae* (30); *Streptococcus faecalis* (30); *Neisseria meningitidis* (30); *Neisseria gonorrhoeae* (30); and *Haemophilus influenzae* (28). Within each species, all strains were unselected and recently isolated from different patients, and may be considered as a representative sample of the microflora found in pathological speci-

mens of a large hospital. Most microorganisms investigated in this study were recently isolated from specimens received in the clinical laboratory of St-Rafaël Hospital of the University of Leuven over a period of 3 months. The *H. influenzae* strains and the gonococci were obtained from a recent collection of S.R. Pattijn (University Antwerpen), and the meningococci were obtained from S. De Mayer-Cleempoel (Laboratory of Hygiene and Public Health, Brussels).

Antibiotics. Standard reference powders of the different antibiotics were obtained from the manufacturers. Piperacillin was supplied by Lederle; ampicillin, amoxycillin, carbenicillin, and ticarcillin by Beecham Laboratories; cephalothin by Eli Lilly & Co.; and gentamicin by Schering Corp. Stock dilutions of the different antibiotics at a concentration of 10 mg/ml were stored in equal portions at −20°C. Twofold serial dilutions were freshly prepared in sterile distilled water, and one part of each dilution was added to nine parts of melted and cooled diagnostic sensitivity test agar (DST agar, Oxoid) for the agar dilution method. For the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determinations in broth, the serial dilutions were made immediatlely in Trypticase soy broth (TSB, BBL).

Susceptibility testing methods. (i) Agar dilution method. The susceptibility of all strains was determined by an agar dilution technique in DST agar. Inocula were prepared by culture in TSB: *Enterobacteriaceae* for 4 h, *Pseudomonas* and staphylococci for 6 h, and streptococci overnight. The cultures were further diluted in TSB and inoculated with an automatic multipoint inoculator, delivering 0.001 ml, to obtain a final inoculum of 10^4 to 10^5 colony-forming units (CFU). For testing *H. influenzae,* the strains were grown overnight in TSB plus 5% Fildes extract (Oxoid). Since the yield of such cultures was between 10^7 and 10^8 CFU/ml, they were inoculated (0.001 ml) without further dilution. *N. gonorrhoeae* was grown on chocolate blood agar and *N. meningitidis* was grown on blood agar for 24 h and then suspended in TSB. These suspensions were less homogeneous, so the inoculum varied between 10^4 and 10^6 CFU. For streptococci and *Neisseria,* the DST agar was supplemented with 10% defibrinated horse blood (heated at 80°C for *N. gonorrhoeae*); for *H. influenzae* the DST agar was enriched with 2% Fildes extract and 0.5% supplement C (Difco). The plates were used on the day of preparation. For *Proteus* strains, *p*-nitrophenyl-β-D-glucoside in a final concentration of 50 μg/ml was added to the medium to avoid swarming. Control drug-free plates were similarly inoculated. After incubation at 37°C for 18 h, the MIC was recorded as the lowest concentration of antibiotic that completely inhibited growth; the presence of three or less colonies was disregarded.

(ii) MIC and MBC in broth. In 25 isolates of *E. coli, K. pneumoniae, Proteus mirabilis, Serratia marcescens,* and *Pseudomonas aeruginosa,* the MIC was also determined in liquid medium with the microtiter system. The organisms were precultured in TSB for 4 h and further diluted in TSB to contain approximately 10^5 CFU/ml. The antibiotic concentrations were prepared in TSB in twofold serial dilutions and distributed in 0.05-ml amounts in the microtiter plates, and then 0.05 ml of the inoculum was added. After 18 h at 37°C, the MIC was determined. All wells containing trace growth or no discernible growth were subcultured on DST agar, using a calibrated loop delivering 0.001 ml. The MBC was defined as the lowest concentration that contained no growth or less than 0.1% of the inoculum. In five isolates of each of the five species, the inoculum effect on the MIC and the MBC was determined, using 10^3, 10^5, and 10^7 CFU of inoculum per ml, respectively.

(iii) Determination of parallel resistance. To evaluate a possible parallel resistance between piperacillin and other antibiotics, additional strains were selected on the basis of their resistance to the antibiotics in question: *E. coli* resistant to ampicillin and/or cephalothin; *K. pneumoniae* resistant to cephalothin and/or gentamicin; *S. marcescens* and *P. aeruginosa* resistant to carbenicillin and/or gentamicin; and indole-positive *Proteus* and *Providencia* resistant to carbenicillin. Isolates were designated as resistant when they were resistant to at least 4 μg of gentamicin per ml, to 8 μg of ampicillin or cephalothin per ml, or to 64 μg of carbenicillin per ml. These selected resistant strains together with the strains of the unselected population sample were retested against piperacillin and the antibiotics in question at twofold increasing concentrations up to 1,024 μg/ml. Since the isolates were tested simultaneously with the same inoculum of each antibiotic, a direct comparison of the MICs is possible for each isolate. Therefore the linear regression lines of the log MICs between piperacillin and each of the other antibiotics were calculated on the whole population, susceptible as well as resistant. A perfect correlation (coefficient $r = 1.00$) cannot be expected even with absolute cross-resistance; therefore, the statistical difference was calculated between the observed r values and a correlation coefficient of at least $r = 0.95$, predetermined as the limit level of perfect parallel resistance, for a population sample of the same size.

Analysis. For calculating the geometric means of inhibition, strains resistant to the highest concentration tested were considered as having an MIC of the next twofold concentration (e.g., >128 μg/ml = 256 μg/ml). For statistical evaluation of the correlation coefficients, the method of R. A. Fisher was used as described by Croxton (1).

RESULTS

MICs in DST agar. The in vitro activity of piperacillin against the common gram-positive cocci, the *Neisseria,* and *H. influenzae* is given in Table 1. *Staphylococcus aureus* (penicillin susceptible) had a piperacillin MIC ranging from 0.12 to 8 μg/ml, but 93% of the strains were inhibited at 2 μg/ml or less. Most strains of *Streptococcus pyogenes, S. agalactiae,* and *S. pneumoniae* were inhibited by 0.12 μg/ml or less. *S. faecalis* was inhibited by between 2 and 4 μg/ml. In comparison, the activity of ampicillin was two- to fourfold higher than that of piperacillin on gram-positive cocci. In contrast, piperacillin was somewhat more effective than ampi-

TABLE 1. *Cumulative percentage of gram-positive cocci, Neisseria, and Haemophilus influenzae inhibited by piperacillin and ampicillin*

MIC (μg/ml)	*Streptococcus pyogenes* (14)[a]		*S. agalactiae* (15)		*S. pneumoniae* (30)		*S. faecalis* (30)		*Staphylococcus aureus* (30)		*Neisseria meningitidis* (30)		*N. gonorrhoeae* (30)		*H. influenzae* (28)	
	PIP[b]	AMP[c]	PIP	AMP	PIP	AMP	PIP	AMP	PIP	AMP	PIP	AMP	PIP	AMP	PIP	AMP
0.015	100	100			80	100				3	100		76	37		
0.03				67	97					13		50	93	67		
0.06				100	100					40		97	93	80	89	
0.12			73						7	53		97	97	93	93	7
0.25			100				33		10	70		97	97	97	100	100
0.5							97		40	93		100	97	100		
1							100		70	93			100			
2									93	100						
4									93							
8									100							

[a] Number of isolates tested.
[b] Piperacillin.
[c] Ampicillin.

cillin against the *Neisseria* and *H. influenzae*, with most isolates inhibited at 0.12 μg/ml or less.

Table 2 shows the activity of piperacillin on gram-negative bacilli as compared with that of ampicillin, carbenicillin, and cephalothin. The activity of piperacillin against members of the *Enterobacteriaceae* was excellent. A concentration of 4 μg/ml inhibited 70% of *E. coli*, 80% of *Salmonella*, 90% of *P. mirabilis*, 80% of the indole-positive *Proteus* and *Providencia*, 46% of *Klebsiella*, 80% of *Enterobacter*, 89% of *Citrobacter*, and 23% of *S. marcescens*. Its activity against *P. aeruginosa* is even more striking: 50% of the isolates were inhibited at 2 μg/ml, 83% at 4 μg/ml, and 97% at 16 μg/ml.

Against *Enterobacteriaceae*, piperacillin was obviously more active than ampicillin (except against *Salmonella*) and cephalothin (except against *Klebsiella* and *Salmonella*). Piperacillin was far superior to carbenicillin against *Pseudomonas*, *S. marcescens*, and *Klebsiella* and at least equally active against the other gram-negative organisms.

This is clearly illustrated by comparing the geometric mean MICs of piperacillin, ampicillin, amoxycillin, carbenicillin, ticarcillin, and cephalothin (Table 3). The largest difference in activity between piperacillin and ampicillin or amoxycillin was observed against indole-positive *Proteus* strains (16 to 32 times). Against *Klebsiella*, *Enterobacter*, *Serratia*, *Providencia*, and *Citrobacter diversus*, piperacillin was four to eight times more active, and against *E. coli* and *Citrobacter freundii* it was only slightly more effective than ampicillin and amoxycillin. In comparison with carbenicillin and ticarcillin, the activity of piperacillin was four to eight times greater against *Klebsiella*, *Serratia*, and *Citrobacter diversus* and twice as great against *P. mirabilis* and the indole-positive *Proteus* strains. There was no advantage of piperacillin over carbenicillin and ticarcillin with regard to activity against *E. coli*, *Enterobacter*, *Salmonella*, and *Providencia*. Cephalothin was more active than piperacillin against *Klebsiella* and *Salmonella*, equally active against *E. coli*, but two to eight times less active against *Enterobacter*, *P. mirabilis*, and *Providencia* and 50 to 200 times less active against indole-positive *Proteus*. The activity of piperacillin against *P. aeruginosa* was 5 to 10 times higher than that of carbenicillin and ticarcillin, whereas ampicillin, amoxycillin, and the cephalosporins were completely ineffective. Also, against the other *Pseudomonas* species, piperacillin was four to six times more active than the other penicillins. Piperacillin had no advantage over the other penicillins against *Acinetobacter*. Piperacillin was more effective

TABLE 2. *Distribution of susceptibilities of gram-negative bacilli to piperacillin, ampicillin, carbenicillin, and cephalothin*[a]

MIC (μg/ml)	Cumulative percentage of isolates inhibited															
	E. coli (60)[b]				*Salmonella* (30)				*P. mirabilis* (61)				Indole-positive *Proteus* + *Providencia* (91)			
	PIP	AMP	CAR	CEF	PIP	AMP	CAR	CEF	PIP	AMP	CAR	CEF	PIP	AMP	CAR	CEF
0.25									79	2	15		47		25	4
0.5						3			85	28	80		68	2	50	5
1			3			73	3	23	88	80	84	31	75	3	61	8
2	32	5	20	5	30	80	63	80	88	84	84	97	78	5	72	9
4	70	45	63	30	80	80	80	83	90	84	84	98	80	10	75	10
8	70	70	70	75	80	80	83	87	92	84	85	98	85	19	76	11
16	70	70	70	88	87	83	83	97	97	85	85	98	86	42	79	15
32	73	70	70	97	87	83	83	97	98	85	88	98	88	65	82	21
64	78	70	70	97	87	83	83	97	100	88	88	98	89	70	82	29
128	83	70	70	97	87	83	83	97		88	95	100	99	81	83	53
	K. pneumoniae (80)				*Enterobacter* (49)				*C. freundii* (15)				*C. diversus* (12)			
	PIP	AMP	CAR	CEF	PIP	AMP	CAR	CEF	PIP	AMP	CAR	CEF	PIP	AMP	CAR	CEF
1	1			12	2			2			7	7				33
2	6			42	59	6	24	6	53	7	67	13				83
4	46			56	80	12	63	8	93	13	87	20	83			83
8	57	2		64	80	31	71	10	100	87	93	27	83			83
16	59	11		81	80	45	73	20		93	100	60	83	17		92
32	61	29		89	86	55	75	39		100		87	83	58		100
64	66	55	9	97	86	69	78	63				100	83	83	17	
128	76	57	29	99	96	71	80	71					92	83	75	
	S. marcescens (30)				*Acinetobacter* (30)				*P. aeruginosa* (60)				*Pseudomonas* spp. (42)			
	PIP	AMP	CAR	CEF	PIP	AMP	CAR	CEF	PIP	AMP	CAR	CEF	PIP	AMP	CAR	CEF
0.25						10			3				14	5	5	
0.5						17	7		3		3		21	9	5	
1	3					17	17		8		5		31	14	7	
2	23				13	17	30		50		5		38	21	9	
4	23		3		30	33	33		83		5		43	21	17	
8	30		27		83	47	47	7	88		5		69	31	24	2
16	30		27		100	87	83	17	97	2	13		83	31	50	7
32	33		27			100	97	20	97	7	58	2	95	57	64	9
64	93	13	27				100	20	97	10	85	2	100	62	64	9
128	100	23	27					57	97	18	97	2		83	67	19

[a] PIP, Piperacillin; AMP, ampicillin; CAR, carbenicillin; CEF, cephalothin.
[b] Number of isolates tested.

TABLE 3. *Geometric mean MICs of piperacillin and other β-lactam antibiotics*[a]

Species	No.	Geometric mean MIC (μg/ml)					
		PIP	AMP	AMO	CAR	TIC	CEF
Gram-positive cocci							
S. aureus	30	0.90	0.15	0.24	1.3	1.8	0.12
S. pyogenes	14	0.02	0.02	0.02	0.06	0.09	0.02
S. agalactiae	15	0.14	0.04	0.04	0.63	1.3	0.08
S. pneumoniae	30	0.02	0.02	0.02	0.25	0.08	0.08
S. faecalis	30	2.1	0.40	0.38	23.7	33.5	17.5
Total	119	0.22	0.08	0.09	1.13	1.82	0.29
Gram-negative cocci and *Haemophilus*							
N. meningitidis	30	0.02	0.05	0.09	0.05	0.04	0.30
N. gonorrhoeae	30	0.02	0.03	0.08	0.06	0.10	0.20
H. influenzae	28	0.03	0.23	0.26	0.20	0.20	2.2
Total	88	0.03	0.07	0.12	0.08	0.09	0.49
Enterobacteriaceae							
E. coli	60	9.4	16.0	17.0	12.4	8.2	8.7
K. pneumoniae	80	19.2	87.4	157.6	197.4	153.5	6.0
Enterobacter sp.	49	5.0	34.4	88.6	10.2	5.9	55.6
C. freundii	15	6.1	8.0	40.3	2.8	1.5	14.6
C. diversus	12	7.6	47.9	101.6	135.6	80.6	2.4
Salmonella sp.	30	6.2	2.8	2.2	5.2	3.6	2.6
S. marcescens	30	24.8	198.5	217.8	99.3	88.4	256
P. mirabilis	61	0.4	1.9	1.9	1.1	.8	1.7
P. vulgaris	30	0.5	35.9	78.8	1.5	1.3	82.5
P. morganii	30	0.4	26.0	99.3	.7	.6	256
P. rettgeri	11	1.7	23.4	60.1	2.6	2.0	38.7
Providencia sp.	20	11.3	46.9	61.8	20.4	16.6	21.9
Total	428	4.08	21.88	35.37	11.04	8.01	14.67
Nonfermenters							
P. aeruginosa	60	3.4	198.5	210.4	37.6	16.4	247.3
Pseudomonas sp.	42	4.1	25.0	29.5	29.5	15.7	184.0
Acinetobacter sp.	30	6.6	6.6	9.0	7.3	3.6	111.4
Total	132	4.11	47.37	55.01	23.98	11.46	187.78

[a] PIP, Piperacillin; AMP, ampicillin; AMO, amoxycillin; CAR, carbenicillin, TIC, ticarcillin; CEF, cephalothin.

than the other broad-spectrum penicillins and cephalothin against *N. meningitidis, N. gonorrhoeae,* and *H. influenzae,* but the MIC values were low for all β-lactam antibiotics. Ampicillin and amoxycillin were the most effective antibiotics against penicillin-susceptible staphylococci, β-hemolytic streptococci, *S. pneumoniae,* and *S. faecalis.* Piperacillin was nearly equally active against β-hemolytic streptococci and *S. pneumoniae,* but five times less effective against the staphylococci and the enterococci. However, its activity on streptococci was still 5 to 15 times higher than that of carbenicillin and ticarcillin.

Susceptibility to piperacillin of strains resistant to other antibiotics. The term "cross-resistance" is generally used in the clinical sense that isolates with an MIC above a concentration limit achievable in vivo for one antibiotic also surpass the useful limit for another, related antibiotic. In the pure microbiological sense, it means that an increase in the MICs of one antibiotic is accompanied by a parallel increase in MICs of another related antibiotic, suggesting the same mechanism of resistance. If two related antibiotics have an intrinsically different degree of activity but a similar degree of bioavailability, there may be some discordance: strains moderately resistant to one antibiotic may still be completely susceptible in the clinical sense to the other. Therefore we prefer to use the term "parallel resistance" to indicate a parallel increase in MIC values.

To evaluate the possible parallel resistance between piperacillin and other antibiotics, additional strains were selected on the basis of their resistance to antibiotics considered to be of first choice against particular species. On these large population samples with isolates distrib-

uted over the entire range of MIC values, the MIC of piperacillin and the corresponding MICs of the antibiotic used for comparison may be plotted on a logarithmic scale, as shown in Fig. 1 for *E. coli*, and the best fit of the regression line can be calculated. The better the parallelism between the MICs of both antibiotics, the more the correlation coefficient (r) will approximate 1.0. The correlation coefficients of the regression lines between piperacillin and other antibiotics in different species are shown in Table 4, together with their statistical evalation.

Ampicillin parallel resistance was tested on 70 *E. coli* and 79 *P. mirabilis* isolates. The high correlation coefficients, $r = 0.959$ and $r = 0.938$, respectively, were not statistically different from $r = 0.95$, defined as the limit level for complete parallel resistance. This demonstrates that parallel resistance exists between piperacillin and ampicillin. The same is true for piperacillin and carbenicillin, as shown by the correlation coefficients in 101 isolates of indole-positive *Proteus* and *Providencia* ($r = 0.945$), in 40 *S. marcescens* ($r = 0.931$), and in 81 *P. aeruginosa* ($r = 0.923$). In contrast, the correlation coefficients of the regression lines between piperacillin and cephalothin or gentamicin were significantly different ($P < 0.001$) from $r = 0.95$: for cephalothin in 70 *E. coli* isolates, $r = 0.589$, in 79 *P. mirabilis* isolates, $r = 0.743$, and in 71 *K. pneumoniae* isolates, $r = 0.897$; for gentamicin in 71 *K. pneumoniae* isolates, $r = 0.512$, in 40 *S. marcescens* isolates, $r = 0.864$, and in 81 *P. aeruginosa* isolates, $r = 0.830$. Therefore a parallel resistance between piperacillin and gentamicin or cephalothin is statistically unlikely, in spite of some relatively high correlation coefficients. This seems to be logical for gentamicin, which belongs to an antibiotic group with a completely different mechanism of action, but it is not immediately evident for the β-lactam antibiotic cephalothin. The apparently parallel increasing resistance between piperacillin and gentamicin in *S. marcescens* and *P. aeruginosa* may be due to "co-resistance." The term "co-resistance" is used to indicate the coincidental appearance of independently evolved resistance of two unrelated drugs, e.g., by transfer of multiple resistance with plasmids. In the case of co-resistance, the MICs of both antibiotics may increase but in an independant way: some strains resistant to one

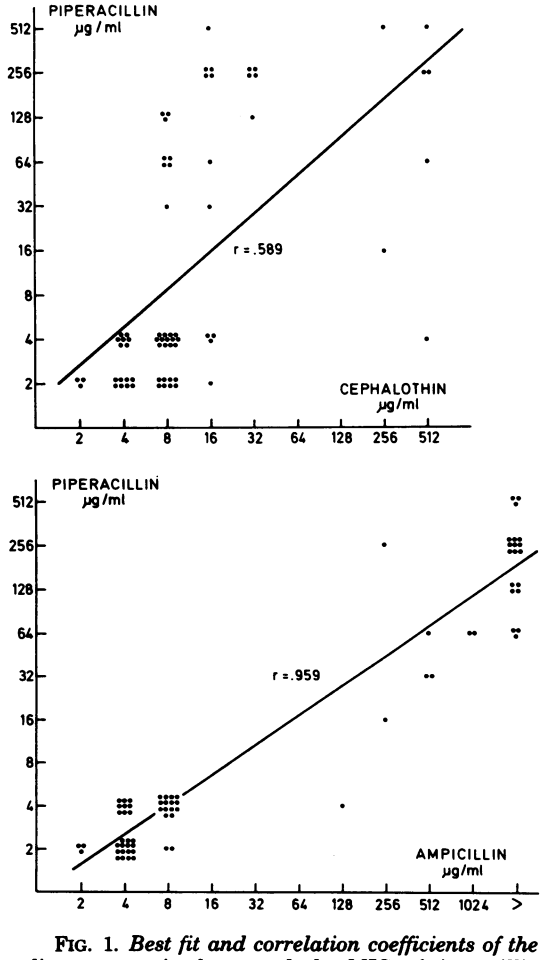

FIG. 1. *Best fit and correlation coefficients of the linear regression between the log MICs of piperacillin and ampicillin or cephalothin in 70 E. coli isolates.*

TABLE 4. *Parallel resistance between piperacillin and other antibiotics expressed by the correlation coefficient of the linear regression between the log MICs*[a]

Species	No.	Comparison of PIP with:	Correlation coefficient	Probability $r \geq 0.95$
E. coli	70	AMP	0.959	NS[b]
	70	CEF	0.589	<0.001
P. mirabilis	79	AMP	0.938	NS
	79	CEF	0.743	<0.001
Indole-positive *Proteus* + *Providencia*	101	CAR	0.945	NS
S. marcescens	40	CAR	0.931	NS
	40	GM	0.864	<0.005
K. pneumoniae	71	CEF	0.897	<0.001
	71	GM	0.512	<0.001
P. aeruginosa	81	CAR	0.923	NS
	81	GM	0.830	<0.001

[a] PIP, Piperacillin; AMP, ampicillin; CEF, cephalothin; GM, gentamicin; CAR, carbenicillin.

[b] NS, r not significantly different from $r = 0.95$ for a given population sample.

antibiotic will remain susceptible to the other and vice versa, and thus the correlation coefficient will differ significantly from the predefined limit of $r = 0.95$. In this way the co-resistance with cephalothin in *E. coli* is easily explained by the fact that 19 of 23 cephalothin-resistant isolates were also resistant to ampicillin. But no significant parallel resistance with piperacillin was demonstrable, since 8 of the 47 isolates susceptible to cephalothin nevertheless had high piperacillin MICs because of their resistance to ampicillin. The situation is perhaps less clear for the lack of parallel resistance between piperacillin and cephalothin in the *Klebsiella* isolates. Although the correlation coefficient ($r = 0.897$) was significantly different from $r = 0.95$, it would not be different if the accepted limit for parallel resistance were defined as $r = 0.90$. It may be concluded that there is a complete parallel resistance in all species tested between piperacillin and ampicillin or carbenicillin, but not with cephalothin or gentamicin. The cross-resistance between ampicillin and amoxycillin and between carbenicillin and ticarcillin is well established. Therefore the conclusion may be extended to the existence of a complete parallel resistance between piperacillin and the known broad-spectrum penicillins. On the other hand, resistance to cephalothin and, by extension, also to other cephalosporins seems to be independent of resistance to piperacillin and vice versa.

MIC and MBC determination in broth. MIC and MBC of piperacillin, ampicillin, and carbenicillin were determined for 25 isolates of *E. coli, P. mirabilis, K. pneumoniae, S. marcescens,* and *P. aeruginosa.* Table 5 shows the results expressed as number of isolates inhibited (MIC) or killed (MBC) at each concentration. With an inoculum of 10^5 CFU/ml, little difference was observed in TSB between the MIC and MBC values. Within the species *E. coli, P. mirabilis, K. pneumoniae,* and *S. marcescens,* the MBCs were generally the same as the MICs or only one concentration higher for all three antibiotics tested. In most of the *P. aeruginosa* isolates, the MBC was one concentration higher than the MIC for both piperacillin and carbenicillin, as reflected also by the difference between the geometric mean MIC and the geometric mean MBC. The geometric mean MICs in TSB

TABLE 5. *Inhibitory and bactericidal effect of piperacillin (PIP), ampicillin (AMP), and carbenicillin (CAR) on microorganisms in broth*

Concn (μg/ml), MIC/MBC	No. of strains inhibited (MIC)/killed (MBC)					
	E. coli (25)[a]			*P. mirabilis* (25)		
	PIP	AMP	CAR	PIP	AMP	CAR
0.12				1/0		
0.25	2/2			14/13		4/2
0.5	7/5			6/8	11/5	19/19
1.0	12/13	3/2		4/4	12/15	1/3
2.0	4/5	18/11	8/6		1/3	
4.0		4/8	12/9		0/1	
8.0		0/4	5/9			1/1
16.0			0/1		1/1	
Geometric mean	0.82/0.89	2.05/2.94	3.68/4.59	0.35/0.38	0.84/1.11	0.51/0.57
	K. pneumoniae (25)		*S. marcescens* (25)		*P. aeruginosa* (25)	
	PIP	AMP	PIP	CAR	PIP	CAR
0.25					1/1	
0.5	1/1					2/0
1.0			2/2		2/0	1/2
2.0	1/0		4/4	1/0	5/3	0/1
4.0	15/12		2/1	3/2	8/7	
8.0	8/10	3/3	1/2	4/3	8/8	1/0
16.0	0/2	4/4		0/3	1/6	1/0
32.0		7/5	2/0			9/6
64.0		8/9	8/8			6/6
128.0		3/4	6/5			4/6
512.0						
1,024.0				1/0		
>1,024.0				16/17		
Geometric mean	4.46/5.42	35.7/38.8	21.7/27.1	294.1/357.0	3.68/5.73	29.4/49.9

[a] Number of strains tested.

TABLE 6. *Inoculum effect on MIC and MBC in five isolates of five different microorganisms*[a]

Organism	Piperacillin				Ampicillin				Carbenicillin			
	10^3 CFU/ml		10^7 CFU/ml		10^3 CFU/ml		10^7 CFU/ml		10^3 CFU/ml		10^7 CFU/ml	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. coli												
1	0.5	1	1	1	2	2	2	4	2	2	4	4
2	0.5	0.5	2	2	4	4	4	8	4	4	8	8
3	1	1	2	2	4	4	4	8	2	4	2	8
4	1	2	8	8	4	4	16	32	8	8	32	32
5	2	2	4	4	4	4	8	8	2	4	8	8
Klebsiella												
1	0.12	0.5	2	2	4	8	64	128	64	64	256	256
2	1	2	8	8	8	8	64	64	64	64	256	256
3	2	4	8	8	32	32	128	256	256	256	1,024	1,024
4	4	16	64	64	32	32	512	1,024	256	256	>[b]	>
5	4	4	16	16	32	32	256	512	256	256	1,024	1,024
P. mirabilis												
1	0.25	0.25	0.25	0.5	0.5	1	2	2	0.5	0.5	1	1
2	0.25	1	0.5	1	1	1	1	2	0.5	0.5	1	8
3	0.25	0.5	0.5	0.5	2	4	4	8	0.5	1	1	2
4	1	1	1	1	1	1	4	4	0.5	1	1	4
5	2	2	64	256	16	32	256	256	8	16	64	64
S. marcescens												
1	1	1	2	2	32	64	512	512	4	4	8	32
2	2	2	2	2	64	64	256	256	8	16	32	64
3	2	4	2	16	64	256	256	512	8	8	16	16
4	2	2	64	64	32	32	64	64	8	8	32	32
5	64	128	512	1,024	>	>	>	>	>	>	>	>
P. aeruginosa												
1	0.12	0.25	0.5	32	1	4	256	1,024	0.25	0.25	0.5	4
2	0.12	0.25	16	256	64	512	1,024	1,024	0.25	0.5	1	16
3	1	2	4	8	128	256	1,024	>	16	32	64	64
4	2	4	8	32	32	32	128	512	32	64	64	256
5	4	4	8	16	32	128	256	1,024	32	64	64	256

[a] MIC and MBC expressed in micrograms per milliliter.
[b] >, More than 1,024 μg/ml.

compared with those in DST agar for the same isolates showed no major differences for *P. mirabilis, K. pneumoniae, S. marcescens,* and *P. aeruginosa* with all antibiotics tested. However, the geometric mean MIC of piperacillin against *E. coli* was substantially higher in DST agar than in TSB (2.78 versus 0.82 μg/ml); the same was true for ampicillin (4.72 versus 2.05 μg/ml), but not for carbenicillin (3.03 versus 3.68 μg/ml).

Inoculum effect. In five selected strains of the species *E. coli, P. mirabilis, K. pneumoniae, S. marcescens,* and *P. aeruginosa,* the effect of the inoculum on the MIC and MBC was studied, using inocula of 10^3, 10^5, and 10^7 CFU/ml. The differences in MIC and MBC between inocula of 10^3 and 10^5 CFU/ml were very small with all species and with all three antibiotics tested. Table 6 shows the MICs and MBCs of each strain with inocula of 10^3 and 10^7 CFU/ml. Within the *E. coli* isolates, the differences in MBC between the inocula were small, never exceeding a factor of four for piperacillin and carbenicillin and with a factor of two to eight for ampicillin. Within the *K. pneumoniae* isolates, the increase in the MBC was two to four times for piperacillin, four to eight times for carbenicillin, and eight to 32 times for ampicillin. Within the *P. mirabilis* isolates, the differences were very variable for piperacillin: three of five strains had the same MBC with the large inoculum and one differed by a factor of two, but in the last strain the increase in MIC was 32 times and the increase in MBC was as great as 128 times with the larger inoculum. For ampicillin and carbenicillin, two- to eightfold differences were noted. Within the *S. marcescens* isolates, the increase in MBC was small (two to eight times) for ampicillin and carbenicillin and more variable (one to 32 times) for piperacillin. Within the *P. aeruginosa* strains, the largest variations in MIC and MBC were observed between the small and large inocula, especially for piperacillin. Two strains that were particularly susceptible with the small inoculum (MBC of 0.25 μg/ml) showed an increase in MBC by factors of 128 and 1,024 with the large inoculum, whereas the same strains had increases of only 8- and 32-fold for carbenicillin. The three remaining strains showed increases in MBC of two- to eightfold for both antibiotics.

DISCUSSION

This study demonstrates that piperacillin has an unusually broad spectrum of antibacterial activity against the gram-negative bacilli (*Enterobacteriaceae,* nonfermenters, *H. influenzae*) as well as the gram-positive and gram-negative cocci. Piperacillin actually combines the spectrum of activity of both ampicillin and carbenicillin and in addition has good activity against *Klebsiella, Enterobacter,* and *Citrobacter.* Furthermore, the activity of piperacillin is at least equivalent, but generally superior, to that of ampicillin and carbenicillin, except against *Salmonella* and the gram-positive cocci, which are slightly more susceptible to ampicillin. However, this is of minor importance for a broad-spectrum penicillin that is destined, in the first instance, for use in life-threatening infections with gram-negative bacilli. In this respect, its activity at low concentrations against *P. aeruginosa* is outstanding. Since the MICs and MBCs are closely related and in most species only slightly influenced by the inoculum, piperacillin will be bactericidal at concentrations very near to the MIC. However, a factor that needs confirmation and further investiation is the large increase in the MBC with the large inoculum (10^7 CFU/ml) in some strains of *P. aeruginosa* that belonged, surprisingly, to the isolates most susceptible to a small inoculum. This study further reveals a complete parallel resistance between piperacillin and both ampicillin and carbenicillin. The term "parallel resistance" is used intentionally instead of "cross-resistance," since resistance and cross-resistance are clinical terms used with regard to their therapeutical consequences. However, the importance of parallel resistance is largely mitigated by the fact that the activity of piperacillin is often a multiple of that of ampicillin or carbenicillin. Since the drug is well tolerated, even in massive intravenous doses, as shown in a pharmacokinetic study (manuscript in preparation), only the isolates highly resistant to ampicillin or carbenicillin will be resistant to piperacillin. Further investigations of piperacillin in clinical conditions seem indicated.

This study was supported by a grant from the Lederle Laboratories, Cyanamid Co., Pearl River, N.Y.

I wish to thank Jos Vendeven and Claire Van Ourti for their excellent technical assistance.

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